



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of) Examiner: Daniel M. Sullivan
Van Der Kooy et al.) Art Unit: 1636
Serial No. 09/966,768)
Filed: September 28, 2001)
For: "Primitive Neural Stem Cells)
and Method for)
Differentiation of Stem)
Cells to Neural Cells")

DECLARATION OF DEREK VAN DER KOOY

I, Derek van der Kooy, declare:

1. I am a named inventor of the subject matter claimed in United States Patent Application No. 09/966,768, filed on September 28, 2001 (hereinafter the '768 application).
2. I attended University of Toronto, where I obtained a Ph.D. in the year 1980 in the area of Anatomy. I am currently a Professor at the University of Toronto. My education and professional experience are described in further detail in my curriculum vitae, a copy of which is attached as Appendix A.
3. I have read and understood the Office Action, dated August 10, 2005 in the '768 application. I note that the Examiner has rejected claims 1-11, 13-17, 20-22, 25-27, 29, 30, 33-38, 41, 42, and 47-49 for allegedly failing to satisfy the enablement requirement of 35 U.S.C. §112, first paragraph. The purpose of this declaration is to provide factual evidence that primitive neural stem cells can be generated from embryonic stem cells of species other than mice by the methods provided in the '768 application.

4. The specification of the '768 application discloses culture conditions and methods to generate primitive neural stem cells. As explained in detail below, the culture conditions and methods of the invention have been used by a researcher working under my direction to successfully plate and culture human embryonic stem cells in minimal, serum-free media such that clonal sphere colonies, called neurospheres, are formed. These clonal sphere colonies comprise primitive neural stem cells and can be differentiated into neurons and glial cells. These data were generated using morphological and immunocytochemical techniques described in the instant specification.

5. Primitive neural stem cells were generated directly from a single human embryonic stem ("HES") cell. The human primitive neural stem cells were capable of being passaged and the cells developed in the absence of embryoid body formation.

6. Single HES cells were plated at low density in serum-free media with LIF, EGF, FGF, heparin, and B27. In these conditions, neurospheres formed that contained a mixture of primitive neural stem cells and their progenitor cells. Single HES cells formed neurospheres with a frequency of 0.01%. Appendix B shows a sphere colony that clonally proliferated in a neurosphere assay from a single HES cell to a sphere size of approximately 150 μ m in 14 days.

7. HES-derived neurospheres expressed neural markers and not mesodermal or endodermal markers. The Appendix B "Nestin (Whole sphere)" photograph shows nestin immunolabelling in a whole outgrowing neurosphere. Appendix C shows RT-PCR analysis of neural and non-neural lineage gene expression in RNA extracted from HES cells, embryoid bodies and clonal primitive neural stem cell neurospheres. The neural precursor marker, nestin, and the neuronal marker, β III-tubulin+ were identified, but not markers of other types of tissue like mesoderm (eg. brachyury) or endoderm (eg. GATA-4 and HNF4- α).

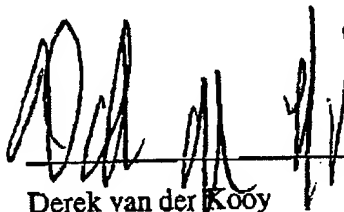
8. The neurospheres can be differentiated to form neurons and glia. Appendix B also shows photographs of cells that were positively immunolabelled as neural precursor cells (nestin), oligodendrocytes (O4+) and glia (β III-tubulin+).

9. The data described above clearly demonstrates the successful isolation of human primitive neural stem cells.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: March 8/06


Derek van der Kooy

Appendix A
CURRICULUM VITAE

DEREK VAN DER KOOY

BIRTHDATE: March 11, 1952

CITIZENSHIP: Canadian

A. 1. ACADEMIC HISTORY

1974-1976	Master's Program, Psychology, University of British Columbia, supervisors: A.G. Phillips and H.C. Fibiger. M.A. awarded, thesis title: Monoamine Substrates of Hippocampal Self-Stimulation
1977-1978	Ph.D. Program, Anatomy, Erasmus Universiteit, Rotterdam, The Netherlands, supervisor: H.G.J.M. Kuypers
1978-1980	Ph.D. Program, Anatomy, University of Toronto, supervisor: T. Hattori Ph.D. awarded, thesis title: The Organization of the Output of the Basal Ganglia in Rats
1980-1980	Postdoctoral Fellow, Cambridge University, England, supervisors: L.L. Iversen and S.D. Iversen
1980-1981	Postdoctoral Fellow, Salk Institute, California, supervisor: F.E. Bloom
1981-1986	Assistant Professor, Department of Anatomy, University of Toronto
1986-1991	Associate Professor, Department of Anatomy, University of Toronto
1991-2002	Professor, Department of Anatomy and Cell Biology, University of Toronto
1995-present	Professor, Institute of Medical Sciences, University of Toronto
1998-present	Professor, Department of Medical Biophysics, University of Toronto
2002-present	Professor, Dept. of Medical Genetics and Microbiology, University of Toronto

2. HONOURS AND AWARDS

Medical Research Council Studentship (1977-1980)
Ham Graduate Award Canadian Anatomical Association (1980)
Richardson Research Fellowship in Anatomy (1980)
Medical Research Council Fellowship (1980-1981)
Medical Research Council Scholarship (1981-1986)
Barr Young Scientist Award Canadian Anatomical Association (1988)
Medical Research Council Scientist (1989-1994)
Medical Research Council Senior Scientist (1995-2000)
Medical Research Council Distinguished Scientist (2000-2005)

3. CURRENT RESEARCH GRANTS

Title of Project	Source	Dates	Amounts
The Role of Cell Lineage in the Development of the Mammalian Forebrain	Canadian Institutes of Health Research	2002-2007	\$159,000/year
Neurobiology of Motivation	Canadian Institutes of Health Research	2004-2009	\$133,000/year
Nicotine Addiction	Canadian Institutes of Health Research	2004-2009	\$100,000/year
Mutational Analysis of Learning and Memory	N.S.E.R.C.	2003-2008	\$38,000/year
Pancreatic Stem Cells	Juvenile Diabetes Research Foundation	2005-2008	\$150,000/year
Stem Cell Plasticity	Stem Cell Network	2003-2008	\$100,000/year

1. BOOK

Smith, C.G. and van der Kooy, D. Basic Neuroanatomy, 3rd edition, Collamore Press, Macmillan Publishing Company, 1985.

2. PUBLICATIONS

Phillips, A.G., van der Kooy, D. and Fibiger, H.C. Maintenance of intracranial self-stimulation in hippocampus and olfactory bulb following regional depletion of noradrenaline. Neuroscience Letters, 4 (1977) 77-84.

van der Kooy, D., Fibiger, H.C. and Phillips, A.G. Monamine involvement in hippocampal self-stimulation. Brain Research, 136 (1977) 119-130.

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3. ABSTRACTS

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- Morshead, C.M. and van der Kooy, D. Adult multipotential, self-renewing neurospheres in vitro are the clonal derivatives of relatively quiescent neural stem cells in vivo. Society for Neuroscience, 2003.
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- Law, E., Nuttley, W.M., van der Kooy, D. A dissociation of olfactory sensation and motivation in *C.Elegans*. Society for Neuroscience, 2004.
- Inoue, T., Coles, B.L.K., Inoue, Y., Bremner, R., Dorval, K., Bessho, Y., Kageyama, R., Hino, S., Matsuoka, M., Craft, C.M., Tano, Y., Roderick, M.R., and van der Kooy, D. Increased OTX2 and decreased Chx10 induce more photoreceptor differentiation from adult human retinal stem cells. Society for Neuroscience, 2004.

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- Karpowicz, P. Runciman, S., Inoue, T., Byers, L., Hitoshi, S., and van der Kooy, D. E-Cadherin maintains neural stem cells. Society for Neuroscience, 2004.

C. 1. COURSES TAUGHT

- ANA 300Y
- Arts and Science Anatomy
 - responsible for lectures, labs, notes and exams on neuroanatomy portion
 - each year since 1983.
- ANA 1009F
- Graduate Course in Developmental Cell Biology
 - developed and continue to teach course
 - each or alternate years 1984-1994
- Medical Neuroscience
- some lectures and all labs each year since 1988
 - 1989 Harry Whittaker Award Honourable Mention (runner up as best 1st year medicine primary lecturer)
- JDB 1025Y
- Joint Development Biology Course
 - developed (with J. Rossant and R. Elinson) and continue to teach course.
 - each year since 1997

2. RESEARCH STUDENTS SUPERVISED

Ph.D.

Tim O'Connor	1983-1988	The Innervation of Cerebral Arteries
Gord Fishell	1984-1989	Pattern Formation in the Striatum
Les Krushel	1985-1990	Cell Adhesion in the Embryonic Brain
Tony Bechara	1985-1991	Neurobiology of Opiate Motivation
Cindi Morshead	1986-1994	Origin of Neural Stem Cells
Norm Kumar	1988-1994	Learning in Nematodes
Tom Jaeger	1989-1994	The Discriminative Effects of Drugs
Karim Nader	1990-1995	The Neurobiology of Motivation
Darlene Skinner	1990-1995	Multiple Learning Systems
Eileen van Vulpen	1991-2000	Embryonic Forebrain Development
Glenn Morrison	1991-2000	Learning in <i>C. Elegans</i>
Vince Tropepe	1995-2000	Neural Stem Cells
Steve Laviolette	1997-2003	Neurobiology of Motivation
Colleen Dockstader	1997-2003	Neurobiology of Motivation
Raewyn Seaberg	1999-2004	Neural Stem Cells
Simon Smukler	2002-present	Stem Cells
Tania Alexson	2004-present	Stem Cells
Phil Karpowicz	2003-present	Stem Cell Ethics

Ryan Ting-A-Kee	2002-present	Neurobiology of Motivation
Eric Law	2002-present	Learning Genes

M.Sc.

Janice Johnston	1987-1989	Heterogeneous Organization of Embryonic Ventricular Zone.
Matthew Goldsmith	1987-1990	The Motor for Sperm Motility.
Steven Leyland	1987-1990	Motor for Mitosis.
Taresa Stefurak	1988-1990	Representation of Motivation in Memory.
Kevin Horgan	1988-1990	Innervation of Cerebral Arteries.
Ray Hoffarth	1990-1993	The Reeler Mutant Mouse
Eileen van Vulpen	1991-1993	The Development of Cholinergic Striatal Neurons.
Joe Wen	1991-1993	Learning Mutants.
Jayson Parker	1991-1993	The Neurobiology of Cocaine Reward.
Glenn Morrison	1992-1994	Learning Mutants
Nirit Bernhard	1997-1999	Learning Genes
Hance Clarke	1996-1999	Multiple Learning Systems
David Martens	1996-2000	Neural Stem Cells
Karen Atkinson	2000-2003	Learning Genes
Mary Sunderland	2001-2003	PAX6 and Retinal Stem Cells
Andrew Heinmiller	2003-present	Neurobiology of Motivation
Brian DeVeale	2005-present	Asymmetric Stem Cell Divisions
Maria Guzman	2005-present	<i>C.Elegans</i> Learning
Radha Chaddah	2005-present	Human ES Cell Differentiation

Undergraduate

Approximately 10 undergraduates have done research projects (ANA 498) under my supervision in the last 5 years.

MEMBER OF GRADUATE SUPERVISORY COMMITTEES

In the last six years, I have been on the supervisory committees of approximately 8 students.

Ph.D. RECLASSIFICATION AND Ph.D. ORAL EXAM COMMITTEES

For approximately 9 additional students in past six years (from Anatomy, Zoology, Medical Genetics, Pharmacology, Dentistry and Psychology).

D. UNIVERSITY ORGANIZATIONS

- 1982-1985 - Organizer and fund raiser for university-wide Neuroscience Lecture Series.
- 1982-1984 - Graduate Recruitment Committee, Department of Anatomy.
- 1990-1992 - Executive Committee - Neurosciences Planning, Faculty of Medicine
- 1989-present - Executive Committee - Graduate Council Department of Anatomy
- 1998-2004 - University Program in Developmental Biology
- 1998-present - University Program in Neuroscience

ORGANIZATIONS OUTSIDE THE UNIVERSITY

- 1985-1989 - Medical Research Council Grants Committee for Neuroscience (Neuroregulatory Mechanisms).
- 1990-1994 - Scholarships Committee Alberta Heritage Foundation for Medical Research.
- 1995-1998 - Medical Research Council "Scientist" Committee.
- 1990-1995 - Human Frontier Science program "Fellowship" Committee
- 1989-1998 - Editorial Board - Pharmacology, Biochemistry and Behavior.
- 1991-present - Editorial Board - Behavioural Brain Research
- 1996-present - Board of Reviewing Editors, Science